Effect of Iron Isomaltoside on Skeletal Muscle Energetics in Patients with Chronic Heart Failure and Iron Deficiency: The FERRIC-HF II Randomized Mechanistic Trial

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ABSTRACT

Background: Iron repletion augments exercise capacity in chronic heart failure (CHF) but there is a lack of mechanistic data explaining how iron could increment exercise performance despite minimal changes in hemoglobin (Hb). Besides Hb, iron is an obligate component of mitochondrial enzymes that generate cellular energy in the form of adenosine triphosphate and phosphocreatine (PCr). Dynamic phosphorus magnetic resonance spectroscopy (^{31}P MRS) is a noninvasive tool that quantifies *in vivo* muscle energetics by measuring the kinetics of PCr recovery after exertion. We tested the hypothesis that intravenous (IV) iron repletion in CHF enhances skeletal muscle energetics as reflected by shorter PCr recovery half-times (PCr $t_{1/2}$) on ^{31}P MRS.

Methods: We enrolled 40 patients (50% anemic) with CHF, NYHA class≥ II, LVEF≤ 45%, and iron deficiency (ferritin<100μg/L or 100-300μg/L with transferrin saturation [TSAT]< 20%). Subjects underwent stratified (anemic vs non-anemic) randomization (1:1) to a single, double-blinded, total dose infusion of Iron Isomaltoside (IIM) or saline placebo with endpoints reassessed early at 2 weeks post-treatment to minimise confounding from exercise adaptation. The primary endpoint was PCr $t_{1/2}$ at 2 weeks. Secondary endpoints included adenosine diphosphate recovery half-time (ADP $t_{1/2;}$ energetic marker), iron status, symptoms, Hb, exercise capacity and safety.

Results: In the total population, treatment groups were similar at baseline. At 2 weeks, IIM improved PCr $t_{1/2}$ (adjusted difference -6.8s [95% confidence interval 11.5,-2.1], P=0.006), ADP $t_{1/2}$ (-5.3s [-9.7,-0.9], P=0.02), ferritin (304ng/mL [217,391], P<0.0001), TSAT (6.8% [2.7,10.8], P=0.002), NYHA class (-0.23[-0.46,-0.01], P=0.04), resting respiratory rate (-0.7 breaths/min [-1.2,-0.2], P=0.009) and post-exercise Borg dyspnea score (-2.0[-3.7,-0.3], P=0.04), but not Hb (2.4g/L [-3.5,8.4], P=0.41). Adverse events were similar between groups. In subgroup analyses, IIM improved PCr $t_{1/2}$ in anemic (-8.4s [-16.7,-0.2], P=0.04) and non-anemic (-5.2s [-10.6,0.2], P=0.06) cohorts.

Conclusion: In patients with CHF and ID, a total repletion dose of IIM given at a single sitting is well tolerated and associated with faster skeletal muscle PCr $t_{1/2}$ at 2 weeks, implying better mitochondrial function. Augmented skeletal muscle energetics might therefore be an important mechanism via which iron repletion confers benefits in CHF despite minimal Hb changes.

Clinical Trial Registration: URL: https://www.clinicaltrialsregister.eu/ctr-search/trial/2012-005592-13/GB. Unique identifier: EudraCT 2012-005592-13.

CLINICAL PERSPECTIVE

What is New?

- Little is known about how intravenous iron repletion augments exercise capacity in chronic heart failure (CHF) despite minimal hemoglobin changes.
- This randomized, double-blind, placebo-controlled Ferric Iron in Heart Failure (FERRIC-HF) II trial shows that a single total dose infusion of Iron Isomaltoside repleted iron stores and augmented skeletal muscle energetics at 2 weeks post-infusion.
- Enhancements in skeletal muscle energetics, which imply better mitochondrial function, were accompanied by improved symptoms despite no change in hemogobin at 2 weeks.

What are the Clinical Implications?

- Augmented skeletal muscle energetics is a likely mechanism via which iron repletion confers benefits in CHF despite minimal hemoglobin changes.
- FERRIC-HF II supports clinical iron repletion in CHF.
- A total repletion dose of Iron Isomaltoside given at a single sitting is feasible in CHF patients.

INTRODUCTION

Iron deficiency (ID) is prevalent and ominous in chronic heart failure (CHF),[1-4] and its correction markedly improves symptoms and exercise tolerance. Yet, in landmark studies such as the seminal FERRIC-HF (Ferric Iron in Heart Failure) trial, the hemoglobin (Hb) response to intravenous (IV) iron was surprisingly small (5 g/L increase over 4 months),[3,4] and did not correlate with changes in exercise indices. Consequently, it is unknown how IV iron augments exercise capacity despite minimal changes in Hb, and this paucity of mechanistic data partly hampers the clinical uptake of iron repletion in CHF.

Exercise is a highly energetic process with skeletal muscle contractions powered by the hydrolysis of adenosine triphosphate (ATP) which is buffered by phosphocreatine (PCr) consumption.[5,6] To sustain exertion, PCr is resynthesised via glycolysis and mitochondrial oxidative phosphorylation (OXPHOS) which generates over 95% of cellular ATP.[7,8] Iron is a component of Hb and myoglobin, and of proteins of the Krebs cycle, ß-oxidation pathway, and the electron transport chain which determine OXPHOS capacity.[9-12] In animal studies, cellular ID triggers mitochondrial dysfunction and exercise intolerance, even in the absence of anemia.[13-15] Thus, iron repletion might enhance exercise capacity by energizing skeletal muscle independently of Hb. To date, no clinical trial has addressed this in humans.

Dynamic phosphorus magnetic resonance spectroscopy (^{31}P MRS) is uniquely suited to quantifying human skeletal muscle energetics *in vivo*.[16,17] It enables the real-time noninvasive tracking of skeletal muscle PCr, ATP and inorganic phosphate (Pi) concentrations and cytostolic pH during exercise and recovery. Because PCr replenishment on cessation of exercise is predominantly driven by OXPHOS,[18] the PCr recovery half-time (PCr $t_{1/2}$) is an inverse marker of mitochondrial oxidative function that is relatively independent of exercise intensity.[16] We conducted the FERRIC-HF II trial to test the hypothesis that iron repletion with a single total dose infusion of Iron Isomaltoside (IIM) would improve skeletal muscle energetics as reflected by a shorter PCr $t_{1/2}$ in CHF patients.

METHODS

Study Design

FERRIC-HF II was an investigator-led, randomized, double-blind, placebo-controlled, mechanistic trial conducted at King's College Hospital, London, UK. The study involved 1 screening visit, 2 baseline visits within 4 weeks of screening, and 2 final visits 2 weeks after treatment allocation. The protocol was approved by the South-Central Berkshire ethics committee, the UK Medicines and Healthcare products Regulatory Agency, and the independent local research governance board. King's College London University and King's College Hospital NHS Foundation Trust were co-sponsors. The King's Health Partners clinical trial office monitored the trial and ensured compliance with the International Conference on Harmonization guidelines for Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from all patients. The trial is registered on clinicaltrialsregister.eu (EudraCT 2012-005592-13) and data from this analysis is obtainable from the authors on reasonable request.

Study Patients

Eligibility criteria were: age ≥30 years; stable symptomatic CHF (New York Heart Association [NYHA] III and left ventricular ejection fraction [LVEF] ≤45%, or if NYHA II then LVEF ≤40% within the preceding 6 months); use of optimal CHF drugs for ≥4 weeks without dose changes for ≥2 weeks; screening Hb <120 g/L in females and < 130 g/L in males (anemic group) or ≥120 g/L in females and ≥ 130 g/L in males (non-anemic group); ID as defined by the FERRIC-HF criteria of ferritin <100 μg/L or 100-300 μg/L with transferrin saturation (TSAT) <20%;[3] folate and vitamin B_{12} levels ≥ lower limit of reference range; resting blood pressure ≤160/100 mm Hg; and a negative pregnancy test in women of child-bearing age.

Exclusion criteria were a history of acquired iron overload, known hemochromatosis or first degree relatives with hemochromatosis; an allergic disorder (e.g., asthma, eczema, and anaphylactic reactions); prior hypersensitivity to IV iron drugs or their excipients; active infection, bleeding, malignancy, hemolytic anemia, rheumatoid arthritis, and myelodysplasia; HIV/AIDS; chronic liver disease with transaminases >3 times the upper limit of the reference range; chronic lung disease with $FEV_1 < 50\%$ predicted; coagulopathy or anticoagulated for a metallic valve or LV thrombus; contraindications to MRS; immunosuppressant use; renal dialysis; need for erythropoietin or blood transfusions; unstable angina; severe obstructive cardiac lesions; uncontrolled arrhythmias; and musculoskeletal limitations.

Randomization

Qualifying patients attended for 2 baseline visits at week 0 collectively including a clinical history, physical examination, NYHA class assessment, 12-lead electrocardiogram, blood tests, Kansas City Cardiomyopathy Questionnaire (KCCQ), echocardiogram, 6-min walk test, cardiopulmonary exercise test, quadriceps muscle ^{31}P MRS, and a vastus lateralis muscle biopsy. At the end of the second visit, patients were randomly assigned within 2 strata (anemic or non-anemic) in permuted blocks of 4 to a single total repletion dose of IIM or placebo (normal saline) in a 1:1 ratio. Randomization was performed by a clinical trials pharmacist using an automated web-based system (Sealed Envelope Ltd, London, UK).

Study Drug and Blinding

Iron (III) isomaltoside 1000 (Monofer®; Pharmacosmos A/S, Holbaek, Denmark) was provided as a solution for IV infusion in 100mg iron/mL ampoules. The total repletion dose was calculated to the nearest multiple of 100mg using the Ganzoni formula: body weight (kg) x 2.4 x (15 - patients Hb[g/dL]) + 500 mg (for stores).[19] Doses of 0-10 mg/kg and 11-20 mg/kg were infused over 30 and 60 minutes respectively. Doses exceeding 20 mg/kg were split and given at 2 separate sittings 1 week

apart. Monofer® was added to 100 mL sterile 0.9% saline for infusions. Patients randomized to placebo had their repletion dose and infusion duration calculated as above but received 100 mL sterile 0.9% saline over the infusion period. All patients were observed for 1 hour post treatment with heart rate and blood pressure documented every 15 minutes. Patients who received placebo were offered Monofer® at the end of the study if they remained iron-deficient.

To achieve blinding, allocated therapy was dispensed by a clinical trials pharmacist to unblinded research nurses who prepared and administered the infusions using opaque IV bags and giving sets (Medipak, Virginia, USA). All other members of the research team vacated the infusion room before the allocated therapy was collected from pharmacy. A curtain shielded the infusion arm from the patient. The unblinded nurse was not involved in assessing endpoints.

Primary Endpoint

The primary endpoint was skeletal muscle energetics at 2 weeks post-treatment as assessed by the PCr $t_{1/2}$ on dynamic ^{31}P MRS. This was performed on a clinical 3T scanner (Achieva, Philips Medical Systems, Best, the Netherlands). Patients were asked to refrain from strenuous exercise and alcohol for the preceding 24 hours, and from caffeine and food for the preceding 6 and 2 hours, respectively. Heights and weights were measured to the nearest integer and used to calculate lean body mass.[20] Subjects were positioned feet-first and supine in the scanner and a 14 cm diameter ^{31}P surface coil (Philips Medical Systems, Best, the Netherlands) was strapped to the dominant quadriceps. A non-ferromagnetic weight, equivalent to 10% of lean body mass, was strapped to the dominant ankle (Figure 1).[17] This load was chosen to target an ~30% exertional fall in PCr from baseline thereby avoiding any significant lowering of pH (<6.8) which prolongs PCr $t_{1/2}$.[21] Participants then practiced knee extensions before full entry into the scanner bore, where they were positioned such that the centre of the ^{31}P coil was at magnet isocenter. A series of triplanar ^{1}H scout images were acquired and used

for automatic shimming to optimize the B_0 field homogeneity. Continuous ^{31}P spectra were then acquired (unlocalised, repetition time 2 sec, echo time 0.35 msec, bandwidth 2000 Hz, excitation flip angle optimized for flip angle and 1024 data points) in conjunction with the exercise paradigm consisting of 1 min rest, 1 min knee extensions at 0.5 Hz, and 5 min recovery. This was repeated to permit two PCr $t_{1/2}$ measurements which were then averaged. The same MRS sequence was acquired with a repetition time of 30 sec (8 averages) to provide a fully relaxed spectrum for the calculation of metabolite concentrations.

^{31}P MRS spectra were analysed using jMRUI v5.2 and quantified using the AMARES algorithm with prior knowledge.[22,23] Absolute concentrations of PCr and P_i were calculated making the standard assumption that resting ATP concentration is 8.2 mmol/L cytosolic water (i.e. mM).[24] Intramuscular pH was estimated from the chemical shift of P_i using the following equation: $pH = 6.75 + \log (\alpha - 3.27/5.69-\alpha)$, where α is the chemical shift of the P_i peak relative to PCr.[25] Free cytosolic ADP concentration was calculated by standard means using the creatine kinase equation: ADP = ATP x creatine / [PCr x H^+ x 1.66 x 10^9 M^{-1}], and assuming that total creatine is 42.5 mM.[24] The PCr $t_{1/2}$ was found by fitting the following monoexponential equation to the PCr recovery data: $PCr(t) = PCr_{initial} + (PCr_{end} - PCr_{initial})(1-e^{-kt})$, where t is the time from the end of exercise, $PCr_{initial}$ and PCr_{end} are the PCr concentrations at the start and end phase of recovery, and k is the recovery rate constant. PCr $t_{1/2}$ is calculated as $\log_e 2/k$. The analogous monoexponential equation was fitted to the ADP recovery data to determine ADP $t_{1/2}$, another inverse marker of skeletal muscle energetics.[16]

Secondary and Safety Endpoints

Secondary endpoints included (1) skeletal muscle energetics as reflected by the ADP $t_{1/2}$ and the intracellular concentrations of high-energy phosphate compounds and pH on dynamic ^{31}P MRS; (2) 6-min walk distance; (3) peak oxygen consumption (VO_2) and the ratio of minute ventilation to CO_2

production on cardiopulmonary exercise testing; (4) symptoms as quantified by the NYHA class, Borg dyspnea score, and fatigue scale (assessed using a 10-point visual analogue fatigue scale, from 1 = no fatigue, to 10 = very severe fatigue); (5) quality of life as assessed by the KCCQ; (6) Hb and iron status (ferritin, TSAT, soluble transferrin receptor); (7) N-terminal pro-B-type natriuretic peptide (NT-proBNP); and (8) LVEF.

The 6-min walk test was performed using a flat, straight, 20-m corridor with turnaround points marked by a chair at each end of the measured course. Patients were verbally encouraged to cover as much ground at their own pace for 6 min and were asked to rate themselves on a Borg dyspnea scale before and after exercising. The same blinded investigator supervised all 6-min walk tests for a specific subject. Cardiopulmonary exercise testing was performed using a modified Naughton treadmill protocol.[26] Maximal exercise capacity was attained if the respiratory exchange ratio was >1.00 or had increased by ≥0.15 from the resting value. All treadmill exercise tests were supervised by blinded cardiac physiologists.

Safety endpoints included (1) adverse events; (2) blood pressure; (3) heart rate; (4) respiratory rate; (5) serum creatinine concentration; (6) serum AST; (7) serum C-reactive protein; and (8) blood pressure and heart rate during treatment infusions.

Statistics

Statistical analyses were prespecified and followed the intention-to-treat principle. Sample size estimates were hampered by a paucity of quadriceps ^{31}P MRS data in patients with CHF. Initial calculations based on a trial using calf ^{31}P MRS,[27] suggested that 40 patients in total would be needed to detect a 30 sec difference in PCr $t_{1/2}$ ($\alpha = 0.05$, $\beta = 90\%$, standard deviation = 24 sec) and cover for drop-outs and missing data. Review of the accruing blinded data suggested that the variance and

223 standard deviation of PCr $t_{1/2}$ were likely to be smaller but that 40 patients in total was still sufficient to
224 detect a more conservative difference of 6 sec with a standard deviation of 5 sec ($\alpha = 0.05$, $\beta = 90\%$).
225
226 The primary analysis was a comparison of PCr $t_{1/2}$ at 2 weeks using an analysis of covariance model
227 with baseline PCr $t_{1/2}$ as covariate. All other continuous endpoints were similarly analysed. Missing
228 data were imputed using the last observation carry-forward method for patients who had a last
229 observation recorded post treatment. For those who did not, the mean value for their treatment group
230 was used for imputations. Sensitivity analyses without imputation and with a *post-hoc* Markov chain-
231 Monte Carlo imputation with 10 iterations were performed. Categorical variables were evaluated using
232 a Pearson's χ^2 test. Baseline and week-2 data are described using appropriate summary measures. The
233 estimates and two-sided 95% confidence intervals (95% CI) for the difference between least-squares
234 means of the two treatment arms are given. All statistical tests were two-sided and we judged a P-value
235 < 0.05 significant. All analyses were carried out using Stata 9.2 (Statacorp, Texas) and Statview 4.5 for
236 Windows (Abacus Concepts, California).
237

RESULTS

Patients and Iron Isomaltoside Administration

Between October 2014 and December 2016, we screened 83 outpatients (Figure 2) and randomized 40 to IIM (n=21) or placebo (n=19). Baseline characteristics were balanced between the two treatment groups (Table 1). All patients received their allocated therapy at a single sitting except for 1 subject who received placebo over 2 sittings. In those randomized to IIM, the mean iron repletion dose was 929±320 mg (11±4 mg/kg). No subject was lost to follow-up and only 1 patient did not attend an end-of-study visit due to hospitalization. Overall, 4.2% of the analysed data was imputed. Sensitivity analyses yielded consistent results.

Primary Endpoint

During dynamic ^{31}P MRS the treatment arms exercised against similar weights (IIM: 5.7±0.7 kg, placebo: 5.5±0.9 kg, P=0.57) and achieved similar degrees of exertional PCr depletion at baseline (IIM: 36±11%, placebo: 34±13%, P=0.52) and at 2 weeks (IIM: 38±13%, placebo: 37±9%, P=0.71). At baseline, post-exercise PCr $t_{1/2}$ was similar in the randomized groups (Table 1). After treatment, PCr $t_{1/2}$ improved (shortened) by 17% (-4±10 sec) in the IIM group and worsened by 7% (3±7 sec) in the placebo arm (Table 2). The primary endpoint, PCr $t_{1/2}$ at 2 weeks, was significantly shorter in patients randomized to IIM with an adjusted between-group difference of – 6.8 sec (95% CI -11.5 to -2.1, P=0.006; Table 2, Figure 3). This remained significant even after *post-hoc* adjustment for both baseline PCr $t_{1/2}$ and Hb (-6.8 sec, 95% CI -11.6 to -2.0, P=0.007) or other variables that appeared unbalanced such as age (-8.0 sec, 95% CI -12.8 to -3.2, P=0.002), and NTpro-BNP (-7.2 sec, 95% CI-10.5 to -1.0, P=0.007). No anemia status by treatment group interaction existed (P=0.44).

Secondary Endpoints

Skeletal Muscle ADP $t_{1/2}$, Metabolite Concentrations and pH

Treatment with IIM significantly improved (shortened) post-exercise ADP $t_{1/2}$ (Table 2) by 45% (-5±9 sec) while it lengthened by 3% (3±7 sec) with placebo. No difference in resting or end-exercise PCr and ADP levels were seen between the groups after treatment (Table 2). Neither the resting nor end-exercise pH differed between the groups after treatment (Table 1 & 2), and only 1 patient at baseline, and no patient at 2 weeks, had a pH ≤ 6.8 during exercise. Possibly due to this limited acidosis, no correlation was seen between exertional pH decrements and the degree of PCr depletion, iron status or the visual analogue fatigue scale. As expected, changes in ADP $t_{1/2}$ correlated with changes in PCr $t_{1/2}$ (r=0.65, P<0.001).

Hemoglobin and Iron Status

Ferritin increased by 83% (327±185 ng/mL) in the IIM group and decreased by 24% (2±27 ng/mL) in the placebo group (Table 2). Similarly, TSAT increased by 29% (8±6%) with IIM and by 4% (2±9%) with placebo. Despite this, Hb remained largely unchanged, being minimally increased by 0.4% (0.6±9 g/L) in the IIM arm, and minimally decreased by 0.8% (-1±14 g/L) in the placebo arm. Changes in ferritin (log transformed; r= -0.37, P=0.02) but not Hb (r=0.17, P=0.30) correlated with changes in PCr $t_{1/2}$.

Symptoms, Exercise Capacity, LVEF and NT-proBNP

Therapy with IIM improved symptoms as reflected by reductions in NYHA class and the post-exercise Borg dyspnea score (Table 2). After 2 weeks, 2 (10%), 19 (90%), and 0 (0%) patients in the IIM group had an improved, unchanged, or worse NYHA class, respectively. By contrast, 1 (5%), 15 (79%), and 3 (16%) patients in the placebo group had an improved, unchanged, or worse NYHA class. There was no difference in the pre-exercise Borg dyspnea score (-0.6, 95% CI -1.5 to 0.2, P=0.12), visual analogue fatigue scale or the KCCQ score (Table 1). Symptomatic improvements were not accompanied by significant changes in LVEF, NT-proBNP, 6-min walk distance, visual analogue fatigue scale (-0.59,

95% CI -1.45 to 0.28, P=0.18), peak VO_2 or the ratio of minute ventilation to CO_2 production (-0.2, 95% CI -3.3 to 2.9, P=0.88) despite both groups attaining similar peak respiratory exchange ratios at baseline (Table 1) and at week 2 (IIM: 1.10±0.10, placebo: 1.05±0.09). Changes in 6-min walk distance related to changes in PCr $t_{1/2}$ (r= -0.33, P=0.04), and peak VO_2 related to PCr $t_{1/2}$ in the IIM (r=-0.57, P=0.007) but not placebo (r=-0.30, P=0.21) group at 2 weeks.

Safety Endpoints

The incidence of adverse events was comparable between the treatment arms with 3 (14%) and 1 (5%) events in the IIM and placebo groups, respectively (P=0.34). In the IIM arm, 1 patient had arthralgia during the infusion, 1 patient noted a mild rash at the venepuncture site 1 day post-infusion, and 1 patient had a serious adverse event (hospitalized 1 week post-infusion with unstable angina needing coronary artery bypass surgery) that was judged unrelated to study drug. No anaphylactic reactions occurred. In the placebo arm, 1 patient reported coryzal symptoms 3 days post-infusion.

Infusions of IIM had no impact on hemodynamics. Systolic blood pressure remained unchanged from pre-infusion (122±17 mmHg) to 15 (123±17 mmHg), 30 (122±17 mmHg), 45 (119±19 mmHg), and 60 (121±15 mmHg) minutes post-infusion (all P>0.05). Diastolic blood pressure and heart rate were similarly stable. At 2 weeks, no differences between the treatment arms were seen for blood pressure, heart rate, and serum creatinine, AST or CRP levels, but IIM was associated with significantly reduced respiratory rates (Table 2).

Subgroup Analysis

Baseline characteristics for the anemic and non-anemic subgroups stratified by treatment allocation are shown in Table 1. Mean iron repletion dose was 1155±221 mg (13±2 mg/kg) in anemic patients and 680±204 mg (9±3 mg/kg) in non-anemic patients. Baseline PCr $t_{1/2}$ was similar in the randomized

groups in both anemic and non-anemic subpopulations. Infusion of IIM significantly improved (shortened) PCr $t_{1/2}$ at 2 weeks in anemic patients. A smaller improvement in non-anemic subjects did not reach statistical significance (Table 3).

DISCUSSSION

FERRIC-HF II sought to illuminate how iron repletion could dramatically augment exercise performance in CHF despite minimal Hb changes. We found that, in iron-deficient patients with CHF and an LVEF ≤45%, a single total dose infusion of IIM safely repleted iron stores and was associated with faster skeletal muscle post-exercise PCr recovery kinetics at 2 weeks, implying better mitochondrial function. Enhancements in skeletal muscle energetics occurred despite no change in Hb, but were paralleled by improvements in symptoms as reflected by reductions in NYHA class and the post-exercise Borg dyspnea score. Augmented skeletal muscle energetics might therefore be an important mechanism via which iron repletion improves functional capacity despite eliciting minimal Hb changes.

Dynamic ^{31}P MRS is uniquely able to quantify *in vivo* skeletal muscle energetics non-invasively. At rest, skeletal muscle pH and phosphometabolite (PCr, Pi, ADP) concentrations are dictated not by ATP supply and demand but by sarcolemmal Na^+-dependent Pi and creatine uptake, Na^+/H^+-antiporter activity, and the net balance of adenine nucleotide breakdown and synthesis.[16] At the onset of exercise, ATP consumption by myosin-ATPases increases ATP demand which is met immediately by local PCr breakdown via the creatinine kinase reaction to regenerate ATP.[7] Sustained exertion is then driven by mitochondrial PCr regeneration via OXPHOS and glycolysis.[5-8] Therefore, during exertion, ATP levels remain largely unchanged due to rapid PCr buffering, PCr declines due to its heightened consumption, pH falls due to elevated glycolysis, and ADP and Pi rise due to increased ATP hydrolysis and PCr consumption. At the end of exercise, PCr consumption and glycolysis cease and PCr levels start to recover at a rate commensurate with mitochondrial oxidative ATP synthesis.[24] Dynamic ^{31}P MRS enables the real-time noninvasive tracking of these biochemical events *in situ*, with ^{31}P MRS measures closely related to peak VO_2 and invasive markers of muscle energetics.[16,28,29] In our cohort, resting pH and phosphometabolite concentrations mirrored those reported in other CHF patients and normal

343 subjects[30-32] and were unaltered by IIM. This implies that the processes dictating resting skeletal
344 muscle indices were unperturbed in our subjects and were not influenced by IIM over 2 weeks. On
345 initiation of mild to moderate exercise in-magnet, skeletal muscle ATP levels remained constant, PCr
346 and pH fell, and ADP and Pi rose in our patients as expected (see Figure 2). That the degree of
347 exertional PCr depletion was similar in the treatment groups implies comparable effort. That only 1
348 patient developed significant intracellular acidosis (exercise pH <6.8), which slows PCr $t_{1/2}$,[21] implies
349 that our exercise paradigm did not evoke significant glycolysis and that our PCr $t_{1/2}$ values likely truly
350 reflected oxidative ATP synthesis capacity.
351
352 To the best of our knowledge, this is the first study to show that iron repletion with IIM augments
353 skeletal muscle energetics as reflected by a 14% relative acceleration of PCr recovery kinetics on
354 dynamic ^{31}P MRS. This represents a substantial benefit. In a randomized trial in CHF patients,
355 Adamapolous *et al*., reported a 5% weekly rate of improvement in PCr $t_{1/2}$ with moderate-intensity
356 exercise training.[33] In a double-blind evaluation of the energetic enhancer perhexiline, Lee *et al*.,
357 documented a 4% weekly rate of improvement in PCr $t_{1/2}$ in CHF patients.[27] In young healthy
358 individuals, 2 weeks of high-intensity exercise training improved PCr recovery by 14% with no change
359 in the untrained control group.[34] Thus, over 2 weeks, a single total repletion dose of IIM augmented
360 skeletal muscle energetics to the same extent as 3 weeks of moderate-intensity exercise training in
361 CHF, 3-4 weeks of perhexiline use in CHF, and 2 weeks of high-intensity physical training in younger
362 healthy individuals. In contrast, Melenovsky *et al.*, very recently found no difference in PCr recovery
363 kinetics 4 weeks after infusing Ferric Carboxymaltose (1g) in 13 CHF patients.[35] This might be because
364 their patients exercised longer (6 mins vs. 1 min) against higher resistance (7kg vs. 5.6±0.8kg) during
365 ^{31}P MRS, leading to greater intracellular acidosis (pH 6.92±0.17 vs 6.98±0.07) which renders PCr
366 recovery kinetics less informative of OXPHOS.[16,18,21] This might also reflect their smaller sample size,
367 lack of randomization and a control group, later reassessment at 4 weeks, or the use of Ferric

Carboxymaltose which uniquely induces hypophosphatemia which could blunt energetic gains from iron repletion.[36-38] Several explanations for our findings can be posited.

Post-exercise PCr $t_{1/2}$ is influenced by skeletal muscle O_2 supply (determined by cardiopulmonary function and Hb), storage (myoglobin), and utilization (mitochondria),[16] which are all modifiable by iron. While we had no pulmonary data, cardiac function and hemodynamics were unaltered by IIM consistent with evidence that O_2 delivery does not limit post-exercise PCr recovery in CHF.[39] Skeletal muscle myoglobin levels are also reportedly normal and exertional myoglobin desaturation in CHF does not restrict skeletal muscle metabolism.[40,41] So, the benefits of IIM are unlikely mediated via O_2 storage. A key mechanistic finding in FERRIC-HF II was our observation that Hb levels did not change with IIM. This accords with data in CHF showing that ID impairs exercise tolerance independently of Hb,[1,2] that iron repletion improves functional capacity despite minimal Hb changes,[3,4] and that such Hb changes do not correlate with increments in exercise performance.[3,4] Although skeletal muscle benefits were greater in our anemic subgroup, this is probably not a function of lower Hb *per se* but may reflect the fact that anemic patients had a poorer iron status than non-anaemics. Thus, IIM likely improved energetics by altering skeletal muscle O_2 utilization.

Mitochondria power cellular processes and mitochondrial dysfunction due to ID might have been mitigated by IIM via numerous mechanisms. This includes acceleration of the electron transport chain which sets the pace for OXPHOS and contains iron-sulfur cluster and heme prosthetic groups.[8,12] Iron is also embedded in enzymes of the Krebs cycle,fatty acid ß-oxidation, and carnitine synthesis,[42] and is active in catalase which maintains a permissive redox environment for efficient OXPHOS activity.[43] In human CHF, myocardial ID is linked to diminished catalase and Krebs cycle enzyme levels.[44] In *in vitro* and animal studies, cellular ID impairs cardiomyocyte and skeletal muscle energetics even in the absence of anemia.[13,14,45] Indeed, in rats with ID anemia, correction of anemia but not ID failed to

improve submaximal exercise capacity (which is determined by oxidative enzymes).[15] In contrast, correction of ID but not anemia improved submaximal exercise performance within 15 hours of iron infusions.[46]

Besides skeletal muscle energetic augmentation by IIM, several other aspects of our study bear special emphasis. First, symptoms were improved by IIM at 2 weeks, with reductions in NYHA class and Borg score corroborated by attenuations in resting respiratory rate (presumably due to improved respiratory muscle energetics). Apart from diuretics, no other contemporary CHF therapy can likely match this pace of symptomatic benefit. Second, this is the first trial to test the utility of infusing a total repletion dose of iron at a single sitting. By 2 weeks, we achieved the same degree of biochemical iron repletion as was achieved by 6 to 12 months in prior studies.[4] Third, FERRIC-HF II is the first to show, in a double-blind placebo-controlled trial, that IIM ameliorates ID in CHF patients.

Our study has limitations. We were underpowered to detect differences in certain clinical indices (e.g., peak VO_2 and 6-min walk distance), in safety endpoints such as rare side-effects of IIM, and in the anemic and non-anemic subgroups. This might explain some of our modest correlations and the near-significant effect of IIM on PCr $t_{1/2}$ in non-anemics which merits further study. We did not measure skeletal muscle blood flow so cannot exclude improved limb perfusion as a contributor to enhanced muscle energetics. However, blood supply does not limit skeletal muscle metabolism in CHF,[39] so it is unlikely that any theoretical increase in perfusion with IIM could influence PCr $t_{1/2}$. We chose a short trial duration to minimize attribution of results to skeletal muscle exercise adaptation, so the energizing effect of IIM might have been greater with a longer trial. No adjustments were made for multiple comparisons and we used the last observation carry forward method for imputations.

FERRIC-HF II has potentially important clinical ramifications. Despite data that ID is common and adverse in CHF,[1,2] and that its correction confers benefits,[3,4] clinical uptake of routine iron repletion, particularly in non-anemic patients, remains poor. One potential barrier to adoption is the lack of mechanistic data explaining how iron could trigger such profound exercise benefits despite minimal changes in Hb. Our findings should therefore go some way to reassure clinicians that the benefits of IV iron are real and appear to have a sound mechanistic underpinning. Second, our data reinforces the importance of routinely checking iron indices in CHF patients, and emphasizes that the treatment target should be iron status and not necessarily Hb. Third, our study suggests that a single total repletion dose of IIM can safely replete iron stores with clinical benefits.

In conclusion, the FERRIC-HF II trial has shown that, in patients with CHF and ID, a total repletion dose of IIM given at a single sitting is well tolerated and associated with faster skeletal muscle post-exercise PCr recovery kinetics at 2 weeks, implying better mitochondrial function. Enhanced skeletal muscle energetics occurs despite no change in Hb, but is accompanied by improved symptoms. Augmented skeletal muscle energetics might therefore be an important mechanism via which iron repletion improves exercise capacity in CHF despite minimal changes in Hb.

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DISCLOSURES

Dr Okonko has received speakers fees and research support from Pharmacosmos, and speakers fees from Vifor. Prof McDonagh has received speakers fees from Vifor and sits on their clinical advisory committee. None of the other authors have conflicts of interest.

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	All patients		Anemic patients		Nonanemic patients	
	Placebo (n=19)	Iron Isomaltoside (n=21)	Placebo (n=9)	Iron Isomaltoside (n=11)	Placebo (n=10)	Iron Isomaltoside (n=10)
Demographics						
Age, years	62±13	70±12	63±15	74±6	61±11	65±15
Male gender, n (%)	13(68)	16(76)	7(78)	11(100)	6(60)	5(50)
Caucasian ethnicity, n (%)	14(74)	17(81)	6(67)	10(91)	8(80)	7(70)
Body mass index, kg/m^2	30±7	29±4	29±6	29±4	30±8	29±5
Ischemic etiology, n (%)	10(53)	11(52)	6(67)	7(64)	4(40)	4(40)
Comorbidities						
Coronary artery disease	11(58)	13(62)	6(67)	9(82)	5(50)	4(40)
Hypertension	13(64)	13(62)	5(56)	8(73)	8(80)	5(50)
Hyperlipidemia	7(37)	7(33)	2(22)	6(55)	5(50)	1(10)
Diabetes mellitus	10(53)	10(48)	6(67)	7(64)	4(40)	3(30)
Atrial fibrillation/flutter	4(21)	6(29)	2(22)	3(27)	2(20)	3(30)
Clinical and quality of life						
LV ejection fraction, %	37±8	37±8	37±8	36±9	37±7	39±7
NYHA class	2.4±0.5	2.5±0.5	2.5±0.5	2.4±0.5	2.5±0.5	2.4±0.5
NYHA class III, n (%)	10(53)	9(43)	4(44)	6(55)	6(60)	3(30)
Systolic BP, mm Hg	122±17	124±16	115±14	125±14	128±17	124±18
Diastolic BP, mm Hg	71±14	73±10	67±11	71±8	75±16	76±12
Heart rate, beats/min	71±11	72±10	67±6	71±6	74±13	72±13
Respiratory rate, breaths/min	16±1	16±1	16±1	16±1	16±1	15±1
KCCQ overall score	53±20	64±23	58±27	66±19	48±13	62±28
Exercise parameters						
Peak VO_2, mL/kg/min	14.3±3.1	15.8±4.3	12.7±3.2	16.0±5.6	15.8±2.2	15.6±2.5
Peak respiratory exchange ratio	1.06±0.11	1.10±0.10	1.05±0.10	1.14±0.10	1.07±0.12	1.07±0.10
CPET exercise duration, s	582±202	627±241	551±252	636±289	610±153	617±191
6 min walking distance, m	313±67	324±79	295±70	299±84	330±64	351±68
Pre-exercise Borg dyspnea score	11±1	11±1	11±1	11±2	11±1	11±1
Post-exercise Borg dyspnea score	15±2	14±3	15±2	15±3	14±2	14±2

Table 1. Baseline characteristics. Data are mean±SD, numbers(%), or median (interquartile range).

	All patients		Anemic patients		Nonanemic patients	
	Placebo (n=19)	Iron Isomaltoside (n=21)	Placebo (n=9)	Iron Isomaltoside (n=11)	Placebo (n=10)	Iron Isomaltosic (n=10)
^{31}P MRS measurements						
Resting PCr, mM	39±4	40±6	39±4	42±7	40±5	38±5
Exercise PCr, mM	26±4	25±6	23±5	25±7	28±3	25±5
Resting Pi, mM	4.3±1.0	4.0±0.7	4.4±1.0	3.9±0.8	4.1±1.1	4.2±0.6
Exercise Pi, mM	18.3±6.8	19.1±7.2	18.8±6.9	20.7±8.8	17.9±7.1	17.3±4.8
Resting pH	7.01±0.04	7.02±0.03	7.01±0.02	7.02±0.02	7.01±0.06	7.01±0.03
Exercise pH	7.00±0.05	6.96±0.10	7.00±0.04	6.97±0.07	7.00±0.06	6.95±0.13
Resting ADP, μM	8±5	8±6	8±6	7±4	8±5	10±8
Exercise ADP, μM	34±12	38±21	40±15	40±26	28±5	35±15
ADP $t_{1/2}$, s	23±6	25±9	24±6	26±12	23±6	24±4
PCr $t_{1/2}$, s	33±9	35±12	36±8	38±14	29±8	31±9
Laboratory measurements						
Ferritin, ng/mL	59(39-79)	34(18-50)	45(26-64)	33(18-48)	77(64-90)	44(28-60)
Transferrin saturation, %	18±10	21±8	12±5	16±7	24±10	25±6
Soluble transferrin receptor, mg/L	4.0±1.5	3.6±0.8	4.6±1.5	3.9±0.6	3.4±1.3	3.4±0.9
Hemoglobin, g/L	128±20	130±15	114±19	119±8	140±11	142±10
Creatinine, μmol/L	108±34	121±39	129±32	138±21	89±24	103±46
Aspartate transaminase, iU/L	22±9	22±8	25±8	21±7	20±10	24±8
C-reactive protein, mg/L	5(1-10)	6(3-9)	12(2-22)	5(3-8)	3(1-6)	6(1-11)
NT-proBNP, pg/mL	462(206-855)	1486(245-2054)	790(291-1944)	2696(423-3907)	316(133-629)	696(245-1900
Treatment						
Diuretics, n (%)	12(63)	14(67)	8(89)	7(64)	4(40)	7(70)
ACE-inhibitor or ARB, n (%)	17(89)	16(76)	7(78)	9(82)	10(100)	7(70)
Beta-blockers, n (%)	16(84)	18(86)	7(78)	10(91)	9(90)	8(80)
Spironolactone, n (%)	12(63)	12(57)	5(56)	5(45)	7(70)	7(70)
Digoxin, n (%)	4(21)	6(29)	3(33)	2(18)	1(10)	4(40)
Anticoagulants, n (%)	3(16)	6(29)	1(11)	3(27)	2(20)	3(30)
Antiplatelets, n (%)	13(68)	13(62)	5(56)	8(73)	8(80)	5(50)

Table 1. Baseline characteristics (continued). Data are mean±SD, numbers (%), or median (interquartile range).

	Placebo (n=19)	Iron Isomaltoside (n=21)	Difference (95% CI)	ANCOVA P-value
Primary endpoint				
PCr $t_{1/2}$, s	36±11	30±7	-6.8(-11.5,-2.1)	**0.006**
Secondary endpoints				
ADP $t_{1/2}$, s	24±9	20±6	-5.3(-9.7,-0.9)	**0.02**
Hemoglobin, g/L	127±14	130±13	2.4(-3.5,8.4)	0.41
Ferritin, ng/mL	57(41-84)	369(232-495)	304(217,391)	**<0.0001**
Transferrin saturation, %	21±9	29±6	6.8(2.7,10.8)	**0.002**
NYHA class	2.6±0.5	2.3±0.5	-0.23(-0.46,-0.01)	**0.04**
6 min walking distance, m	324±67	347±72	15(-10,40)	0.24
Pre-exercise Borg dyspnea score	8±2	7±1	-0.6(-1.5,0.2)	0.12
Post-exercise Borg dyspnea score	15±2	12±3	-2.0(-3.7,-0.3)	**0.02**
Peak VO_2/kg, mL/kg/min	14.9±3.5	16.8±4.7	0.5(-1.0,1.9)	0.54
KCCQ overall score	55±24	68±17	12.7(-7.7,33.2)	0.18
LV ejection fraction, %	39±8	41±7	2.2(-1.1,5.6)	0.19
NT-proBNP, pg/mL	334(180-827)	1623(281-2453)	289(-461,1040)	0.44
Resting PCr, mM	40±5	40±6	-0.2(-3.5,3.0)	0.89
Exercise PCr, mM	26±6	25±6	-0.3(-3.6,3.0)	0.86
Resting Pi, mM	3.8±1.1	4.0±1.0	0.3(-0.3,0.8)	0.33
Exercise Pi, mM	18.2±7.2	17.6±6.7	-0.9(-5.0,3.2)	0.66
Resting pH	7.01±0.3	7.01±0.3	-0.01(-0.02,0.01)	0.44
Exercise pH	6.99±0.06	6.97±0.07	0(-0.04,0.04)	0.97
Resting ADP, µM	7±5	8±5	0.9(-2.0,3.8)	0.52
Exercise ADP, µM	34±15	37±18	1.2(-8.1,10.5)	0.80
Safety endpoints				
Systolic blood pressure, mm Hg	119±14	127±12	7.1(-0.3,14.5)	0.06
Diastolic blood pressure, mm Hg	72±13	74±10	0.7(-5.5,6.8)	0.83
Heart rate, beats/min	74±12	71±10	-2.9(-8.8,3.1)	0.34
Respiratory rate, breaths/min	16±1	15±1	-0.7(-1.2,-0.2)	**0.009**
Creatinine, µmol/L	103±38	106±41	-3(-26,20)	0.80
Aspartate transaminase, iU/L	22±6	30±26	8(-4,21)	0.21
C-reactive protein, mg/L	4(2-8)	6(2-11)	0.4(-3.0,3.9)	0.79

Table 2. Endpoints for total population

	Placebo	Iron Isomaltoside	Difference (95% CI)	ANCOVA P-value
Anemic patients	*n=9*	*n=11*		
PCr $t_{1/2}$, s	38±12	30±9	-8.4(-16.7,-0.2)	**0.04**
ADP $t_{1/2}$, s	24±9	18±7	-6.3(-13.5,0.9)	0.08
Hemoglobin, g/dL	117±12	124±6	5.5(-2.7,13.8)	0.18
Ferritin, ng/mL	45±25	456±161	413(295,530)	**<0.0001**
NYHA class	2.7±0.5	2.5±0.5	-0.3(-0.6,0.1)	0.09
Non-anemic patients	*n=10*	*n=10*		
PCr $t_{1/2}$, s	35±11	31±5	-5.2(-10.6,0.2)	0.06
ADP $t_{1/2}$, s	24±9	21±5	-4.4(-9.6,0.9)	0.10
Hemoglobin, g/dL	135±9	137±15	0.5(-8.3,9.3)	0.72
Ferritin, ng/mL	67±24	274±149	176(68,284)	**0.003**
NYHA class	2.6±0.5	2.2±0.4	-0.2(-0.6,0.1)	0.23

Table 3. Endpoints for anemic and non-anemic subgroups

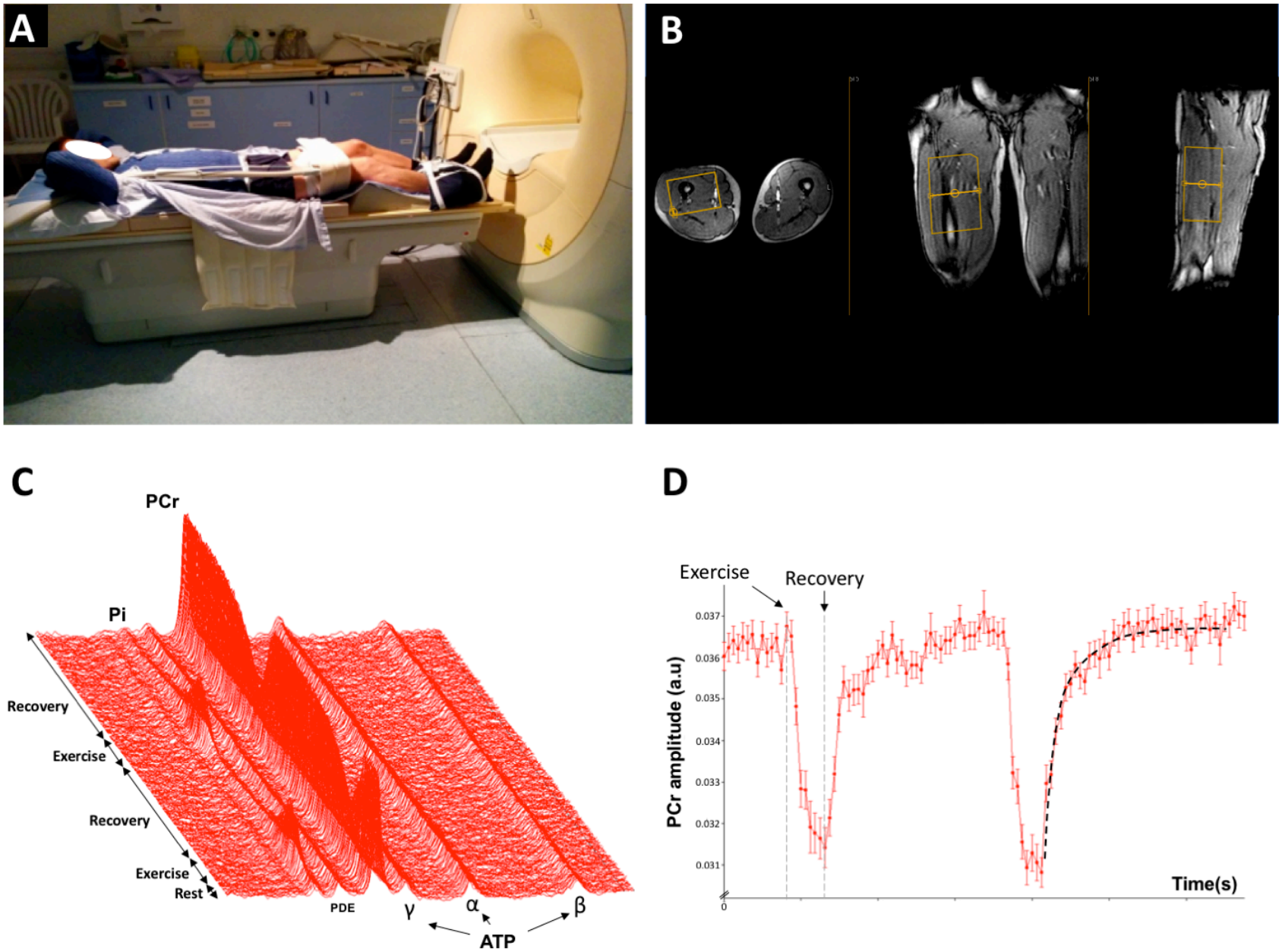

Fig. 1. Dynamic Quadriceps ^{31}P Magnetic Resonance Spectroscopy (^{31}P MRS). The figure shows the experimental setup and example data from multiple studies. Experiments are performed with subjects supine (A) and ^{31}P coil and leg weight attached prior to entry into the magnet bore. ^{1}H scout images of quadriceps are used to optimize positioning (B). ^{31}P MRS data are shown as stacked plot of all phosphometabolite changes (C) and a timecourse of phosphocreatine signal (D) acquired during two bouts of exercise and recovery; PCr $t_{1/2}$ is calculated from the recovery curve (interrupted line) shown in D. PDE = phosphodiester.

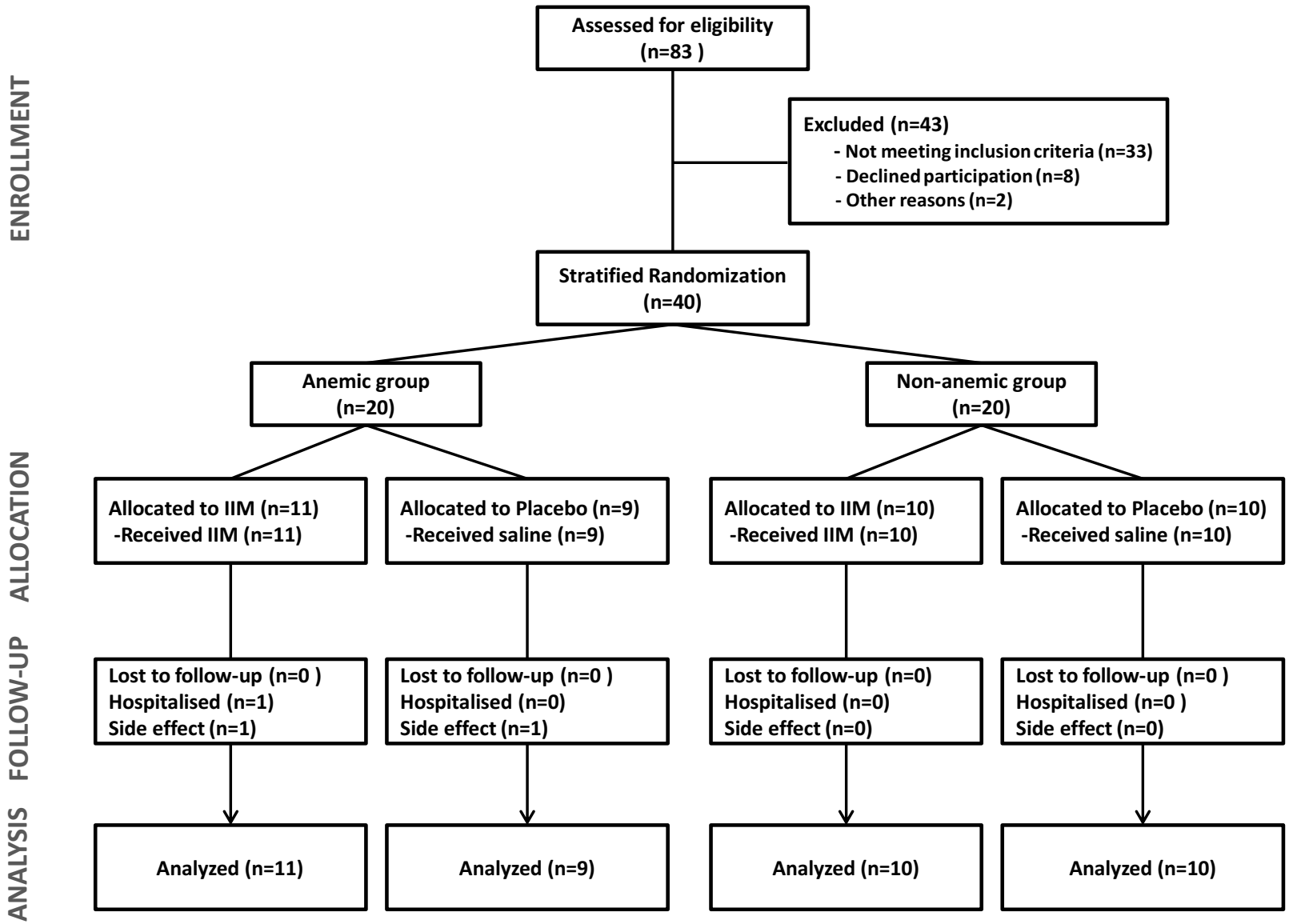

Fig. 2. FERRIC-HF II CONSORT Diagram. Patient disposition during the trial.

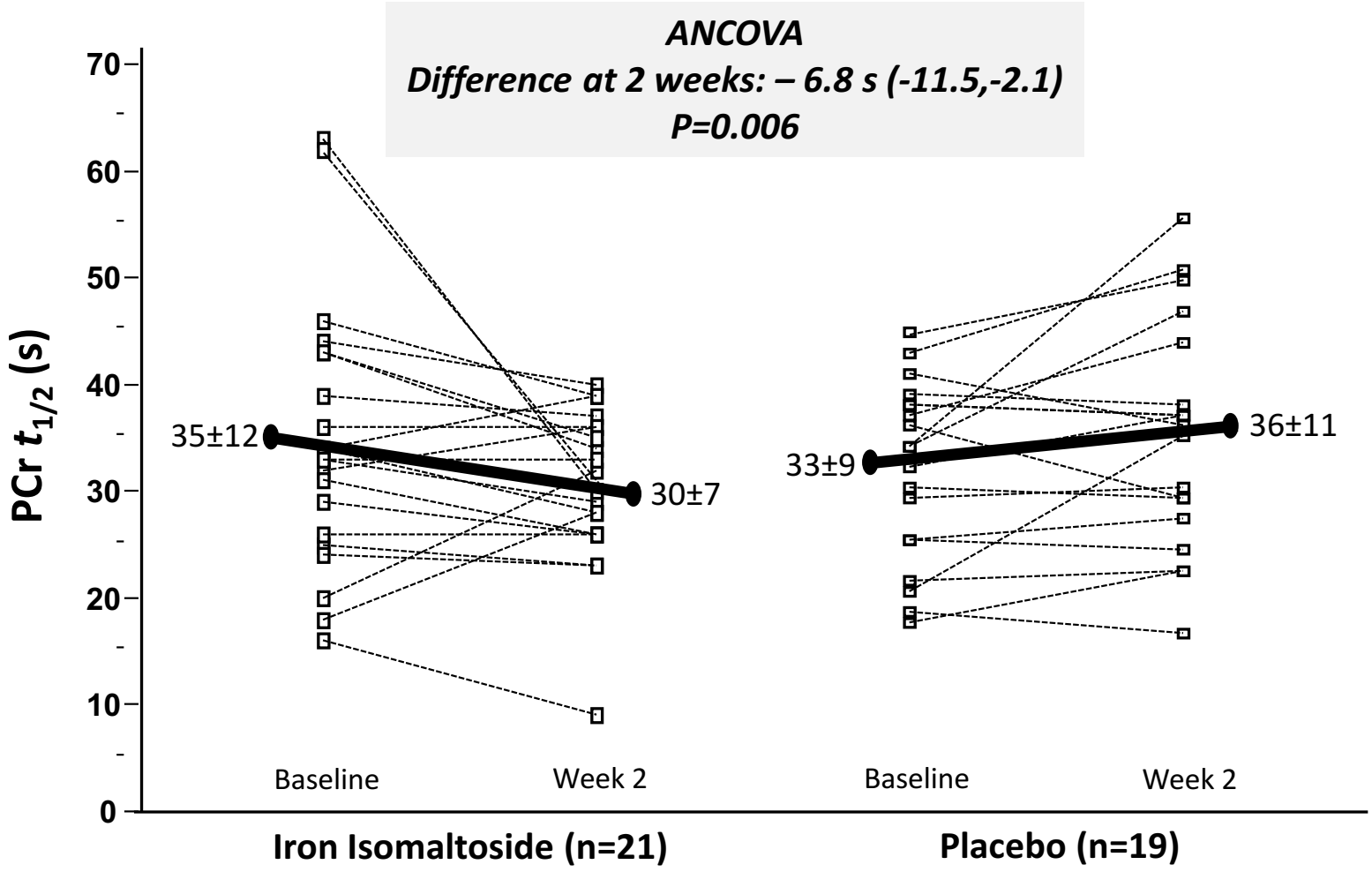

Fig. 3. Primary Endpoint. Individual changes in PCr $t_{1/2}$ with Iron Isomaltoside and saline placebo in total cohort.